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Advanced Paternal Age and Sperm DNA Fragmentation: A Systematic Review

Daniel C. Gonzalez, Jesse Ory, Ruben Blachman-Braun, Sirpi Nackeeran, Jordan C. Best, Ranjith Ramasamy

Department of Urology, Miller School of Medicine, University of Miami, Miami, FL, USA

Purpose: Male ageing is often associated with defective sperm DNA remodeling mechanisms that result in poorly packaged chromatin and a decreased ability to repair DNA strand breaks. However, the impact of advanced paternal age on DNA fragmentation remains inconclusive. The aim of the present systematic review was to investigate the impact of advancing paternal age (APA) on DNA fragmentation.

Materials and Methods: We conducted a thorough search of listed publications in Scopus, PubMed, and EMBASE, in accordance with the PRISMA guidelines.

Results: We identified 3,120 articles, of which nineteen were selected for qualitative analysis, resulting in a sample of 40,668 men. Of the 19 articles evaluating the impact of APA on DFI% (DNA fragmentation Index) included, 4 were on Normozoospermic and subfertile men, 3 on normozoospermic, Oligoasthenoteratozoospermic and Teratozoospermic, 6 on fertile and infertile men, 4 on just infertile men, and 2 evaluated a general population. Seventeen of the ninrnteen studies demonstrated APA's effect and impact on DFI%.

Conclusions: Although there was no universal definition for APA, the present review suggests that older age is associated with increased DFI. In elderly men with normal semen parameters, further studies should be performed to assess the clinical implications of DFI, as a conventional semen analysis can often fail to detect an etiology for infertility.

Keywords: Aging; DNA fragmentation; Paternal age; Sperm parameters

INTRODUCTION

The average age at which couples first reproduce has increased significantly in recent decades, with the mean age now at around 30 years in many countries [1-3]. Since the 1980s, United States birth rates have increased 40% for men 35 to 49 years old and have subsequently decreased 20% for men less than 30 years old [2]. Increased life expectancy, modern societal expectations pressures, and advanced age of marriage has resulted in the tendency for couples to delay parenthood. The increased accessibility to assisted reproductive technology (ART) has increased the chance of older couples to conceive children, hence increasing the average paternal age at first childbirth. While increasing maternal age is well established as a risk factor for adverse reproductive outcome and offspring fitness, the influence of paternal age on sperm parameters and

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Correspondence to: Daniel C. Gonzalez https://orcid.org/0000-0003-1387-7904
Department of Urology, Miller School of Medicine, University of Miami, 1150 NW 14th St, #1551, Miami, FL 33136, USA.
Tel: +1-305-243-7200, **Fax:** +1-305-243-6090, **E-mail:** Ramasamy@miami.edu

fecundity is unclear [4,5].

There's a preponderance of evidence reporting an age-related decline in semen volume, motility, and proportion of morphologically normal sperm [3,6-13]. Additionally, compared with fertile men, infertile men exhibit poor sperm chromatin integrity and *in vivo* fertilizing capability. One study which examined 277 normozoospermic men identified a significantly higher DNA fragmentation Index (DFI) percentage in older (>40 years) men compared to that of younger men [14]. Conversely, Winkle et al [15], found no significant associations with male age, DNA fragmentation, and semen parameters. The mechanisms responsible for age-dependent patterns of DNA fragmentation are not fully understood, but oxidative stress and inefficient apoptosis are thought to be important contributors [16,17].

One intrinsic difficulty with attempting to summarize data for advanced paternal age (APA) is that there is no clearly accepted universal definition of APA. Given that the current population mean for paternal age is 27, the most frequently utilized criterion for APA is more than 40 years of age [18]. While the general consensus is that APA tends to be associated with a decline in semen quality, as well as an increase in DNA fragmentation [10,16], the suggested data appears to be mixed. Further complicating the study is the lack of a universally accepted definition for an abnormal DNA fragmentation threshold, and standard assays. Therefore, we carried out a systematic review to better elucidate the effect of APA on DNA fragmentation by evaluating both age and DNA fragmentation as continuous variables, rather than using cut-offs. Utilizing data from 19 articles (40,668 subjects) we conducted a systematic review to summarize the impact of increasing paternal age on DNA fragmentation.

MATERIALS AND METHODS

1. Methods

A prospective systematic literature review, inclusion and exclusion criteria, and outcome measurement were prepared a priori according to the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines (Supplement File) [19]. This systematic review was accepted in the International Prospective Register of

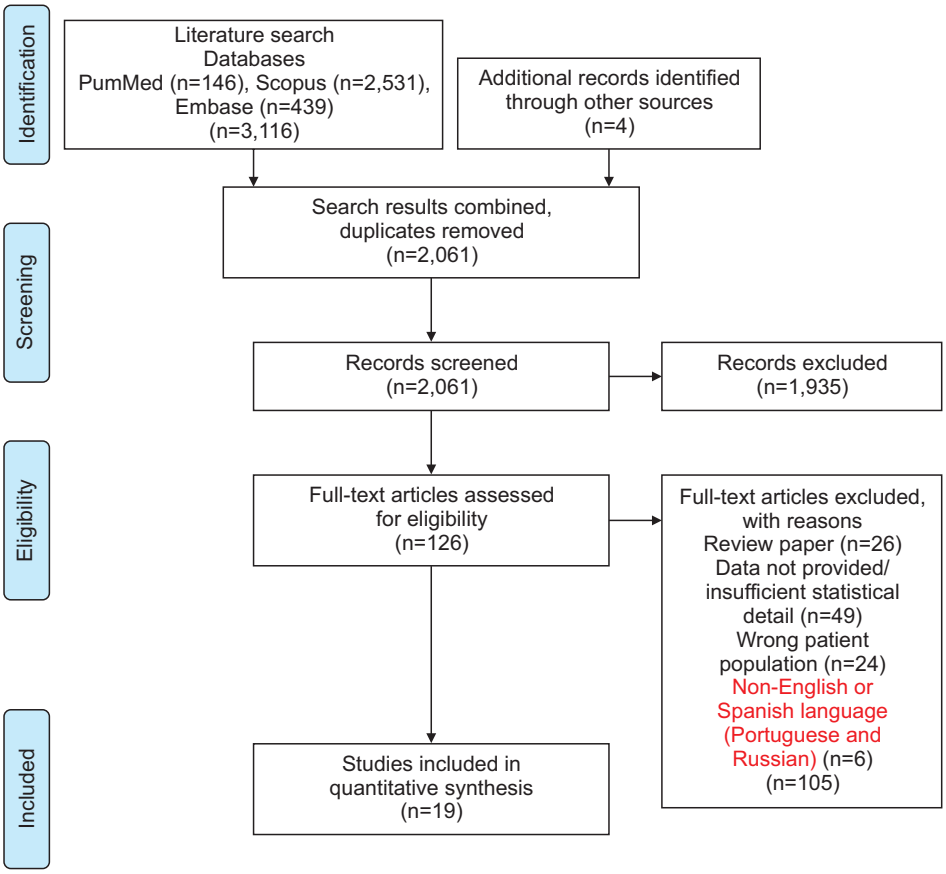

Fig. 1. Flow diagram of search and selection strategy in a systematic review on the impact of advancing paternal age on sperm DNA fragmentation Index.

Systematic Reviews (CRD42020191371) before the commencement of the study, which ensured the transparency of the review process and originality of this study.

2. Data sources and search strategy

A systematic search of Scopus, PubMed, and Embase electronic databases was conducted to identify the relevant studies from inception up until March 2020. We conducted the search by using the search string in [All field] setting: "((((((age OR aging)) AND ((sperm OR semen))) AND ((male))) AND ((fertility OR fertile))) AND ((DFI OR DNA fragmentation))". Additional bibliography lists of retrieved original articles and review papers were manually searched for additional relevant references. We utilized the preferred reporting items for systematic review and meta-analysis checklist (PRISMA) while conducting this study (Fig. 1).

3. Study selection: inclusion and exclusion criteria

Inclusion criteria were original research articles in English and Spanish language addressing the relationship between paternal age, semen parameters, and DNA fragmentation. The search was restricted to studies in humans. Considering the type of participants, studies that assessed DNA fragmentation in fertile men with normal semen analysis in addition to men with a diagnosis of infertility or subfertility were considered, from oligozoospermia (when total sperm count was less than 15 million per mL) to severe oligospermia (when total sperm count was below 5 million per mL). Men who had an underlying varicocele or conditions such as diabetes and obesity were included. This analysis included prospective or retrospective comparative studies which examined the association between age and DNA fragmentation as measured by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labelling (TUNEL), the sperm chromatin structure assay (SCSA), the single-cell gel electrophoresis (Comet) assay, and the sperm chromatin dispersion (SCD) test.

Azoospermic men were not considered eligible and reports including testicular DNA fragmentation were excluded. Articles that were not in English or Spanish language were excluded. Due to the influence of the abstinence period on DNA fragmentation, we excluded articles that did not clearly state the abstinence period. Men who underwent interventions (*e.g.*, chemotherapy, radiotherapy, antioxidants, *etc.*) were excluded from the analysis. Men who were diagnosed with a malignancy and performed a semen analysis before treatment were excluded. Additionally, case reports, editorials, review articles, articles for which the full text could not be found, animal experimental studies, and articles for which the data were not extractable were excluded. Lastly, articles studying the influence of factors such as senescence, environmental pollutants, and cryptorchidism were excluded.

4. Data extraction

Two researchers (D.G and J.B) performed the systematic review and independently extracted data from all selected articles. The opinion of a third observer (J.O) was sought to gain consensus, in the event of any discordance on selecting studies. Extracted data included on study design, publication year, population characteristics, inclusion and exclusion criteria, population, DNA fragmentation assay, main outcomes and conclusions, adjusted results, and statistical methods. The primary outcome of the study was DNA fragmentation evaluation, and volume, concentration, motility, progressive motility, and vitality were considered secondary outcomes.

5. Quality assessment

Each study was scored for their relevance and methodological quality by using the QUADAS 2 (Quality Assessment of Diagnostic Accuracy Studies 2) checklist [20]. Furthermore the following characteristics of the studies were taken into consideration: study population and DNA fragmentation assay.

RESULTS

1. Eligible studies of systematic review

The systematic search retrieved a total of 3,120 articles: 3,116 were identified utilizing the search strategy and four additional articles were identified by manually searching relevant references. After removing duplicates, we were left with 2,061 potentially relevant articles (Fig. 1). The screening for study inclusion was performed in two stages: titles and abstracts were screened in the first stage, and full manuscripts of the articles identified as relevant in the initial screening were retrieved and read in detail for the second stage. After first stage screening, 1,935 articles were excluded,

Table 1. Characteristics of studies included into systematic review

Reference (author, year, nationality)	Population characteristic	Sample size	Age range (y)	Sperm DFI %	Method used	Volume (mL)	Concentration ($\times 10^6$/mL)	% Motility	Progressive motility (%)	Vitality (%)	p-value
Colasante et al, 2019, Brazil [21]	NORMO and subfertile	3,124		5 IQR (2–10)	TUNEL	3±1		60±10			
		775	<35	r=-0.17[a]		-	-	-	-	-	p<0.001
		1,110	36–40	r=-0.16[a]		-	-	-	-	-	p<0.001
		1,239	>41	r=-0.18[a]		-	-	-	-	-	p<0.001
Moskovtsev et al, 2006, USA [22]	NORMO and subfertile	1,125			SCSA				-	-	
		57	<30	15.2±8.4[a]		3.2±1.6	55.5±49.2	35.3±13.7[a]	-	76.7±7.9[a]	[a]p<0.001 when compared to >45
		386	30–34	19.4±12.1[a]		3.3±1.5	57.6±57.4	33.5±17.1[a]	-	71.7±11.5[a]	
		406	35–40	20.1±10.9[a]		3.2±1.5	60.7±59.4	33.4±16.7[a]	-	71.5±11.9[a]	
		187	40–44	26.4±16.0[a]		2.9±1.4	61.9±62.1	29.8±17.7	-	65.5±16.8	
		89	>45	32.0±17.1		2.9±1.8	65.5±65.7	24.9±15.3	-	62.5±14.6	
Brahem et al, 2011, Tunisia [23]	NORMO and subfertile	190			TUNEL						
		10	20–29 NORMO	10.5±1.4		2.8±0.9	95.7±22.9	53.7±4.4	-	-	NS r=-0.094 p=0.516
		16	30–39 NORMO	10.3±4.3		2.8±0.9	93.0±29.4	51.3±6.8	-	-	
		14	40–49 NORMO	9.9±4.0		2.4±0.3[b]	100±46.2	48.1±5.1	-	-	
		10	50–70 NORMO	9.0±5.6		2.0±0.1[b]	99.1±31.3	47.5±3.5	-	-	
		11	20–29 Subfertile	26.2±13.4		3.2±1.6	62.0±40.9	26.3±17.0	-	-	NS r=0.08 p>0.05
		79	30–39 Subfertile	27.6±15.8		3.3±1.5	76.0±59.8	29.0±15.8	-	-	
		40	40–49 Subfertile	30.4±16.8		2.0±1.3[b]	97.9±86.1	25.5±14.2	-	-	
		10	50–70 Subfertile	31.6±18.0		2.4±1.1	98.1±61.3	22.0±17.4	-	-	
Guo et al, 2020, China [24]	NORMO and subfertile	654			SCD						
		71	<30 NORMO	13.0±7.4		-	75.7±42.9	68.5±11.6	61.7±10.9	-	p=0.006 DFI & age for NORMO
		83	30–35 NORMO	14.0±8.6			81.0±51.3	63.3±15.5	56.6±14.3	-	
		71	>35 NORMO	17.5±10.2[a]		-	82.2±44.3	62.5±12.6[a]	55.6±11.7	-	
		135	<30 Subfertile	14.5±11.4			58.3±51.4	52.8±21.6	46.9±20.0	-	p=0.001 DFI & age for subfertile
		124	30–35 Subfertile	16.0±11.0		-	58.6±46.5	47.0±21.2	41.0±19.8[a]	-	
		170	>35 Subfertile	19.8±13.9[a]		-	65.5±64.2	45.7±20.5[a]	39.5±18.8[a]	-	

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Table 1. Continued 1

Reference (author, year, nationality)	Population characteristic	Sample size	Age range (y)	Sperm DFI %	Method used	Volume (mL)	Concentration (×10^6/mL)	% Motility	Progressive motility (%)	Vitality (%)	p-value
Petersen et al, 2018, Brazil [25]	Fertile & infertile	2,178			TUNEL	2.7±1.4	74.6±61.6	63.7±15.7	56.9±16.2	65.0±14.5	
		852	<35	14.7±8.3[a]		-	-	-	-	-	r=0.10
		1,014	36–44	15.9±8.7		-	-	-	-	-	p=0.002
		312	>45	16.2±8.4[a]		-	-	-	-	-	
Kaarouch et al, 2018, Morocco [26]	Fertile & infertile	83			TUNEL						
		42	<40	25		2.6±1.1	24.3±2.9	33	-	60	p<0.05
		41	>40	41[b]		2.4±1.5	18.4±2.6	26	-	55	
Cohen-Bacrie et al, 2009, France [27]	Fertile & infertile	1,653			TUNEL						
		688	36.6±6.1	0–20		-	-	32.6±49.6	32.3±20.7	74.3±13.0	p=0.001
		463	37.1±6.4	20–30		-	-	30.0±52.0	28.6±18.4	71.5±12.5	
		286	38.2±6.8	30–40		-	-	23.7±40.4[a]	25.3±16.8	68.9±12.8	
		216	39.3±9.6	>40		-	-	16.6±28.5[a]	21.3±16.9	62.3±14.8	
Evenson et al, 2020, USA [28]	Fertile & infertile	25,262			SCSA	-	-	-			
		2,000	20–29	12.6±9.7		-	-	-	-	-	
		14,000	30–39	15.2±11.3		-	-	-	-	-	
		8,000	40–49	19.5±14.0		-	-	-	-	-	
		1,000	50–59	26.8±17.4		-	-	-	-	-	
		262	60–80	39.3±21.7		-	-	-	-	-	
Antonouli et al, 2019, Germany [29]	Fertile & infertile	150	43.4±5.5	r=0.23[b] p=0.046	SCD		49.5 r=0.01 p>0.05	54.9+19.2[a] r=-0.29 p=0.012	32.6±17.3[b]	-	
Blachman-Braun et al, 2020, USA [30]	Fertile and infertile	550	37.7±6.5	12.7 [7.8–20]	SCSA	3.0±1.4	43.8±36.3	47.79±10.5			p≤0.001 DFI & age
		38	< 30	11.9±7.9		3.3±1.3	34.5±33.7	46.3±19.8	-	-	
		348	30–40	13.8±9.4		3.2±1.5	44.4±41.6	49.2±18.3	-	-	
		145	40–50	21.2±16.5[a]		2.6±1.2	46.9±38.4	43.9±19.6	-	-	
		19	>50	29.5±16[a]		2.4±1.3	37.5±30	39.3±19.9	-	-	
Alshahrani et al, 2014, USA [31]	Infertile	839		19.9 ± 15.3	TUNEL	3.1 ± 1.5	-	44.7 ± 19.7	-	-	
		69	<30	16.7 ± 11.2		3.4 ± 1.5	42.0±50.5	44.3 ± 14.4	-	-	p<0.005 when >40 compared to all groups
		298	31–40	19.1 ± 14.6		3 ± 1.4	36.6±39.7	45.2 ± 19.5	-	-	
		367	<40	18.7 ± 14. 1		3.1 ± 1.7	42.6±41.5	45.0 ± 18.6	-	-	
		105	>40	24.4 ± 18.5[b]		3.1 ± 1.7	43.8±52.3	43.5 ± 22.9	-	-	

Table 1. Continued 2

Reference (author, year, nationality)	Population characteristic	Sample size	Age range (y)	Sperm DFI %	Method used	Volume (mL)	Concentration ($\times 10^6$/mL)	% Motility	Progressive motility (%)	Vitality (%)	p-value
Nijs et al, 2011, Belgium [32]	Infertile	278			TUNEL						NS
		135	<34	22.4±11.8			40.7±35.8	52.5±18.8	-	-	r=-0.006 p=0.95
		96	35–39	23.35±12.3			40.7±34.2	50.2±16.9	-	-	r=0.027 p=0.79
		47	>40	24.5±12.6			41.5±31.7	57.38±13.0	-	-	r=-0.149 p=0.32
Vagnini et al, 2007, Brazil [33]	Infertile	508			TUNEL						
		186	<35	15.7±10.7[b]		-	-	-	-	-	p=0.034
		140	36–39	18.2±11.3[b]		-	-	-	-	-	p=0.022
		182	>40	18.3±11.0		-	-	-	-	-	p=0.93
Lu et al, 2018, China [34]	Infertile	1,010			SCSA			45.39±19.2			
				r=0.115 p<0.001[a] OR: 0.865 (95% CI, 0.785–0.954) p=0.004		r=0.145 p<0.001[a] OR: 0.548 (95% CI, 0.39–0.77) p=0.001	r=-0.115 p<0.001[a] OR: 0.978 (95% CI, 0.962–0.994) p=0.008	r=-0.487 p<0.001 OR: 0.985 (95% CI, 0.907–1.609) p=0.713	32.29±12.9	-	
Pino et al, 2020, Chile, [35]	General public	2,678			SCD						
		119	21–30	OR: 1							p=0.029 DFI & men over 50
		1,579	31–40	OR: 1.243 (95% CI, 0.364–4.240); p=0.728		OR: 0.821 (95% CI, 0.464–1.450); p=497	OR: 0.987 (95% CI, 0.576–1.690);	-	OR: 3.241[b] (95% CI, 1.175–8.940); p=0.023	-	
		852	41–50	OR: 1.38 (95% CI, 0.39–4.82); p=0.606		OR: 1.332 (95% CI, 0.749–2.369); p=0.328	OR: 1.188 (95% CI, 0.685–2.060)	-	OR: 5.243[a] (95% CI, 1.892–14.526); p=0.001	-	
		128	>50	OR: 4.58[b] (95% CI, 1.167–17.99)		OR: 2.20 (95% CI, 1.11–4.34); p=0.022	OR:1.188[a] (95% CI, 0.685–2.060)	-	OR: 11.911[a] (95% CI, 4.045–35.073); p<0.0001	-	

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Table 1. Continued 3

Reference (author, year, nationality)	Population characteristic	Sample size	Age range (y)	Sperm DFI %	Method used	Volume (mL)	Concentration ($\times 10^6$/mL)	% Motility	Progressive motility (%)	Vitality (%)	p-value
Wyrobek et al, 2006, USA [36]	General public	88			Comet SCSA						
		19	20–29	12.9±7.7		-	-	-	-	-	r=0.72
		20	30–39	16.3±9.6		-	-	-	-	-	p<0.001
		16	40–49	23.2±14.9[a]		-	-	-	-	-	
		17	50–59	35.4±18.6[a]		-	-	-	-	-	
		16	60–80	49.6±17.3[a]		-	-	-	-	-	
Das et al, 2013, Canada [37]	NORMO and OAT	277			SCSA						
		107	<40 NORMO	12±8		-	95±68	-	61±14	-	p=0.008
		41	>40 NORMO	17±13[a]		-	99±58	-	58±17	-	
		97	<40 OAT	12±10		-	35±37	-	30±16	-	p=0.003
		32	>40 OAT	20±18[a]		-	33±37	-	24±14	-	
Plastira et al, 2007, Greece [38]	NORMO and OAT	110			TUNEL						
		26	24–34 NORMO	6.5±1.9		3.3±0.7	51.0±22.4	56.5±4.4	-	-	r=-0.105 p=0.472
		23	35–54 NORMO	5.9±1.7		3.4±0.8	42.0±14.0	54.7±4.1[a]	-	-	r=-0.206 p=0.155
		30	24–34 OAT	26.3±5.3[a]		3.2±1.0	3.3±2.3	23.9±9.6	-	-	r=0.558 p<0.001
		31	35–54 OAT	33.7±6.7[b]		2.6±1.0[a]	5.0±2.7	19.2±8.0	-	-	r=-0.294 p=0.022
Rosiak-Gill et al, 2019, Poland [39]	NORMO and teratoozo-spermic	336			TUNEL						
		116	<40 NORMO	12.7±9.4		3.8±1.7	68.0±50.5	52.4±12.9	-	-	p=0.005
		44	>40 NORMO	17.6±10.4[a]		3.3±1.8[a]	72.4±46.3	56.2±13.6	-	-	
		132	<40 Teratoozo-spermic	13.9±10.9		3.3±1.5	32.6±32.7	30.3±18.5	-	-	p=0.0010
		44	>40 Teratoozo-spermic	21.8±15.1[a]		2.9±1.6[a]	38.6±42.1	31.6±20.4	-	-	

Values are presented as number only, mean±standard deviation, or median [interquartile range].
NORMO: normozoospermia, IQR: interquartile range, TUNEL: terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labelling, SCSA: sperm chromatin structure assay, NS: not significant, SCD: sperm chromatin dispersion test, DFI: DNA fragmentation Index, OR: odds ratio, CI: confidence interval, Comet: the single cell-gel electrophoresis assay, OAT: Oligoasthenoteratozoospermic.
[a]p<0.001, [b]p<0.05.

and 126 articles were identified to assess the full text for eligibility. After this second stage of screening, 105 articles were excluded for reasons shown in (Supplement Table).

2. Characteristics of included studies and comparison of outcomes

The main characteristics of the present systematic review included 19 studies as summarized in Table 1. There were four studies that examined the impact of APA on DNA fragmentation between Normozoospermic and subfertile males [21-24]. Three of the four studies showed a significant difference ($p<0.01$) in favor of APA increasing DNA fragmentation, even among normozoospermic males [21,22,24]. All six studies examining DNA fragmentation between fertile and infertile males with proven primary or secondary infertility, which reported a significant difference in favor of increasing DNA fragmentation with APA [25-30]. Four studies examined males with proven primary or secondary infertility [31-34]. Two studies examined the effect of APA on DNA fragmentation within a general population of healthy males [35,36]. Two studies examined DNA fragmentation between normozoospermic and oligoasthenoteratozoospermic males [37,38] and one study examined teratozoospermic males [39]. Six of the nineteen studies showed a significant difference ($p<0.05$) in favor of APA decreasing sperm motility [22,24,27,29,34,35]. Moreover, in this review three studies examined and noted the incidence of varicoceles, which there was no demonstrated effect of varicocele presence on DNA fragmentation, irrespective of APA [25,31,33]. Five studies showed a significant difference in favor of APA decreasing semen volume [23,34,35,38,39] and two studies demonstrating APA decreasing concentration [34,38]. Out of the 19 studies, 2 failed to show an association with APA and DNA fragmentation [23,32].

Fig. 2 and Table 2 show the scores on overall risk of bias and concerns regarding applicability in this systematic review according to QUADAS 2. For about half of the studies the patient population examined a mix of infertile and fertile patients and hence was judged to be at "high risk" of bias for QUADAS 2 domain "patient selection". Studies were at high risk of applicability concerns in domain "reference standard" when the patient's age category threshold is not comparable to the thresholds of other studies. Overall, the domain "index test" was considered to be at "low risk" because there were 10 studies that utilized TUNEL assay [21,23,25-27,31-33,38,39], 6 studies utilizing SCSA [22,28,30,34,35,37], 1 study using Comet assay [36], and 2 studies that utilized the SCD to quantify DNA fragmentation [24,29].

DISCUSSION

The effect of paternal age on semen quality and

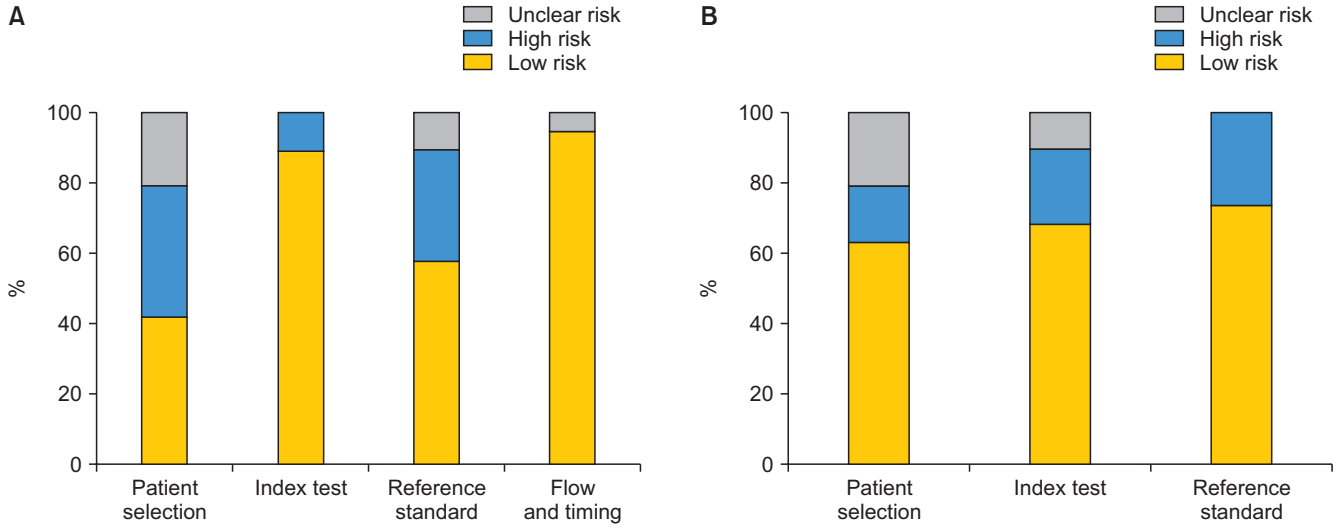

Fig. 2. Overall risk of bias in systematic review. This figure illustrates the overall risk of bias in the systematic review. (A) Proportion of studies with low, high, or unclear risk of bias (%). (B) Proportion of studies with low, high, or unclear concerns regarding applicability (%).The vertical axis represents the number of studies included. The color of the bars represents the risk of bias.

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Table 2. Study characteristics according to QUADAS II recommendations to report the risk of bias for patient selection and the concerns for applicability of data collected in manuscripts eligible for the systematic review

Reference (author, year)	Risk of Bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Colasante et al, 2019 [21]	Low	Low	High	Low	Low	Unclear	High
Moskovtsev et al, 2006 [22]	Low	Low	Low	Low	Low	Low	Low
Brahem et al, 2011 [23]	Low	Low	Low	Low	Low	Low	Low
Guo et al, 2020 [24]	Low	Low	Low	Low	Low	Low	Low
Petersen et al, 2018 [25]	Unclear	Low	Low	Unclear	Unclear	Unclear	Low
Kaarouch et al, 2018 [26]	Unclear	High	Low	Low	Unclear	Low	Low
Cohen-Bacrie et al, 2009 [27]	Low	Low	High	Low	Low	Low	High
Evenson et al, 2020 [28]	Unclear	Low	Low	Low	Unclear	Low	Low
Antonouli et al, 2019 [29]	Unclear	Low	High	Low	Unclear	Low	High
Blachman-Braun et al, 2020 [30]	Low	Low	Low	Low	Low	Low	Low
Alshahrani et al, 2014 [31]	High	Low	Low	Low	Low	Low	Low
Nijs et al, 2011 [32]	High	Low	Low	Low	Low	Low	Low
Vagnini et al, 2007 [33]	High	Low	Low	Low	High	Low	Low
Lu et al, 2018 [34]	High	Low	Unclear	Low	High	Low	Low
Pino et al, 2020 [35]	Low	Low	Unclear	Low	High	Low	Low
Wyrobek et al, 2006 [36]	Low	High/low[a]	Low	Low	Low	High/low[a]	Low
Das et al, 2013 [37]	High	High	High	Low	Low	High	High
Plastira et al, 2007 [38]	High	High	High	Low	Low	High	High
Rosiak-Gill et al, 2019 [39]	High	High	High	Low	Low	High	Low

QUADAS: Quality Assessment of Diagnostic Accuracy Studies.
[a]High risk for Comet (the single cell gel electrophoresis assay), low risk for sperm chromatin structure assay (SCSA).

DNA fragmentation remains controversial. We hypothesized that APA would be associated with an increase in DNA fragmentation. We performed a systematic review comparing DNA fragmentation in different age groups among normozoospermic, subfertile, and infertile men. Our review included data on 40,668 subjects extracted from nineteen available published articles. In the majority of the articles assessed, (17/19) APA was associated with significant increase in DNA fragmentation. Conversely, two articles demonstrated that APA did not influence DNA fragmentation [23,32]. The overall quality of papers demonstrating an association were overall higher than the papers showing no association. Overall, it appears that the majority of articles utilizing SCSA and SCD assays reliably showed an association with APA and increased DNA fragmentation.

The implications of this systematic review are important because the average age of men having children has increased [2]. DNA fragmentation is not a part of a standard infertility workup and in the presence of a normal semen analysis, often no further workup is done [40,41]. However, if DNA fragmentation rates truly do increase as men age, perhaps clinicians should increase their threshold to consider this test in the older men with infertility. In addition, couples pursuing *in vitro* fertilization (IVF) are typically in an older age bracket, and there are several interventions which can improve DNA fragmentation and may improve IVF outcomes in men with high DNA fragmentation [42,43].

In this review, both studies that did not find an effect of APA on DNA fragmentation utilized the TUNEL assay. Several assays are currently available to assess DNA fragmentation, and these assays can be broadly categorized into two types. The first category includes assays where DNA fragmentation is quantified directly by incorporating probes at the site of damage, which detect actual DNA strand breaks. TUNEL, *in situ* nick translation (ISNT), and Comet assay belong to this category. Conversely, the second category includes assays such as SCD test and SCSA, which utilize the property of fragmented DNA to aid denaturation under certain conditions [44]. SCD is based on the ability of intact DNA deprived of chromatin proteins to loop around the lysed and acid treated sperm nuclear

membrane carcass, thus indirectly measuring the susceptibility of DNA to denaturation. Probe incorporation in TUNEL depends on the amount of chromatin that is partially freed from the proteins protecting the DNA, thus it is possible that existing breaks are not detected due to chromatin compaction.

With all systematic reviews, there are limitations that should be taken into consideration. An important weakness of most studies relating to DNA fragmentation and paternal age is that the patient populations are highly selective (*i.e.*, infertile men). The vast majority of studies were retrospective, and therefore the possibility of confounding variables influencing the results cannot be ruled out. It is important to mention that when investigating a paternal age effect on DNA fragmentation, there may be a residual confounding by the presence of varicoceles [45]. Factors such as infertility duration, varicocele, and environmental factors were not reported in several studies. Despite these limitations, our review included data from >40,000 males, and to our knowledge, represents the first formal attempt to thoroughly assess the available data on effects of age in males attending an infertility clinic. Similarly reported by Johnson et al. [10], this review found that only two studies demonstrated the effect of APA on declining sperm concentration, while a majority of studies (6/19) demonstrated APA decreasing semen volume. This review and Johnson et al.'s review [10] supports the association of APA with DNA fragmentation. This review is unique in that we recorded the method of measurement, and were able to determine how direct *vs* indirect assays supported the association of APA and DNA fragmentation. After systematically collecting the information of published articles, a meta-analysis was not amenable given the heterogeneity of the reports as there was not a not consistent cut-off point defining APA among authors. Although there is no validated cut-off points to define APA based on DNA fragmentation data with fertile and infertile men as well in those with normal and abnormal semen parameters, several authors favor for using >40 years as a cut-off point to refer to APA [14,18,26,31,39]. This definition still needs to be further validated in the context of DNA fragmentation, such an analysis requires making many assumptions about the variation in both age structure and traits across different populations. Despite the limitation of the present study and need of future prospective clinical trials that help validate our observations, we believe that analyzing DNA fragmentation in men with APA starting around the age of 40 years can provide an additional tool to set expectations and counsel couples seeking fertility.

CONCLUSIONS

This study suggests a trend to support the effect of APA on DNA fragmentation. As sperm quality is a pivotal factor in fertility potential and ART outcomes, physicians should consider assessing DNA fragmentation in men around the age of 40 years. Given the significant methodological weakness and design of the included studies, future prospective studies are required to investigate the effects of aging on infertile men with normal semen parameters, as a conventional semen analysis can often fail to detect an underlying etiology for infertility.

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Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: DCG, RR. Data curation: DCG, RR, JCB, JO. Formal analysis: DCG, RR, JCB, RBB, SN. Funding acquisition: None. Investigation: DCG, SN. Methodology: DCG, RR, RBB. Project administration: RR, RBB. Resources: RR. Software: RBB, SN. Supervision: DCG, RR, JCB. Validation: DCG, RBB, JO, RR. Visualization: DCG, RR, RBB, JCB. Writing – original draft: DCG, RR, JO, JCB. Writing – review & editing: JCB, DCG, JO, RR.

Supplementary Materials

Supplementary materials can be found *via* https://doi.org/10.5534/wjmh.200195.

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